

## Comparison of *Guizotia abyssinica* Seed Extract (Birdseed) Agar with Conventional Media for Selective Identification of *Cryptococcus neoformans* in Patients with Acquired Immunodeficiency Syndrome

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**Growth of *Cryptococcus neoformans* from the sputum of patients with acquired immunodeficiency syndrome may be obscured by oral contamination with *Candida albicans* on conventional media. We prospectively compared direct plating of sputum and urine onto birdseed agar and compared birdseed agar plating with plating onto Mycosel and Sabouraud dextrose agar cultures. Thirty-two sputum and three urine specimens were compared. *C. neoformans* was isolated from five specimens. In two specimens, one of sputum and one of urine, *C. neoformans* was detected only on the birdseed agar plate because of overgrowth on the conventional media by *C. albicans*. *C. neoformans* produced dark colonies on birdseed agar, unlike *C. albicans*, which produces white colonies. The use of birdseed agar as the primary culture medium for sputum and urine specimens from patients with acquired immunodeficiency syndrome increases sensitivity for *C. neoformans*.**

*Cryptococcus neoformans* is the most frequent cause of invasive fungal disease in patients with acquired immunodeficiency syndrome (AIDS). Incidence figures of cryptococcosis vary but range from 2 to 9% in the United States (D. W. Denning, R. M. Tucker, J. S. Hostetler, S. Gill, and D. A. Stevens, in H. Vanden Bossche, D. W. R. Mackenzie, G. Cauwenbergh, E. Drouhet, B. Dupont, and J. Van Cutsem, ed., *Mycoses in AIDS Patients*, in press; C. R. Horsburgh, Jr., and R. M. Selik, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents. Chemother., abstr. no. 564, 1988) to over 30% in Africans in Europe (5). The most devastating and common form of the disease is meningitis, which carries a markedly reduced life expectancy in patients with AIDS when compared with all other opportunistic infections in these patients (Horsburgh and Selik, 28th ICAAC). Early diagnosis and treatment of less life-threatening forms of the disease, such as pulmonary disease, could lessen the impact of the disease for any given individual. Pulmonary cryptococcosis has been reported for only a small number of patients with AIDS (12) despite the fact that lungs are probably the major route of entry of the organism (6). This low frequency may be artifactual in that conventional fungal sputum cultures from these patients frequently yield *Candida albicans* because of oral contamination of the specimens, since both oral *C. albicans* colonization and disease (thrush) are extremely common in patients with AIDS (5). The colony morphologies of *C. albicans* and *C. neoformans* may be similar on conventional primary isolation media in the first day or two of growth. In addition, *C. albicans* may occur in greater numbers on the plate, thereby obscuring the presence of *C. neoformans* colonies.

Early data suggested that identification of *C. neoformans* from sputum might be enhanced by direct plating onto birdseed agar (10). We have conducted a prospective and

comparative study of the utility of this method for specimens from patients thought likely to have pulmonary or urinary cryptococcosis.

In the period from 1 September 1988 to 15 February 1989, specimens of urine or sputum from patients with AIDS for whom there was a high clinical suspicion of *C. neoformans* infection were submitted to our laboratory with the request "cryptococcus screen." Specimens were handled in the usual way for fungal culture except for additional plating onto birdseed agar (*Guizotia abyssinica* creatinine agar; BBL, Cockeysville, Md.). Sputa were plated directly with a sterile loop or rayon-tipped swab onto Sabouraud dextrose agar and Mycosel agar slants (BBL) and a birdseed agar plate. Urine was centrifuged for 15 min at 1,500 × g, and the sediment was recovered (0.25 ml) with a sterile transfer pipette and evenly distributed over Sabouraud dextrose and Mycosel agar slants and a birdseed plate. All slants and plates were incubated in ambient air at 30°C for 4 weeks with daily inspection. Colonies growing on Sabouraud dextrose or Mycosel agar were Gram stained, and if yeast forms were observed, a germ tube test (2) was done. Yeast colonies that were negative in the germ tube test or produced brown-pigmented colonies on birdseed agar were identified by conventional methods (2) which included examination of morphology on cornmeal agar, a urease test, determination of pigment production on birdseed agar, and determination of sugar assimilation (API yeast system, Analytab Products, Plainview, N.Y.; and Uni-Yeast-Tek system, Remel, Lenexa, Kans.).

The results of these cultures are displayed in Table 1. For *C. neoformans*, the sensitivity and specificity on birdseed agar were both 100%, whereas on Sabouraud dextrose agar slants, the sensitivity was 60% and the specificity was 100%. For *C. albicans* on birdseed agar, the sensitivity and specificity were 90 and 100%, respectively, whereas on Sabouraud dextrose agar, both were 100%. In both instances

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TABLE 1. Isolation of *C. neoformans* and *C. albicans* by conventional fungal culture compared with direct plating onto birdseed agar

Specimen source	No. of specimens with indicated result on:	
	Routine media <sup>a</sup>	Birdseed agar
Sputum		
Positive for <i>C. albicans</i>	9	9
Positive for <i>C. neoformans</i>	2	3
Negative	21	20
Urine		
Positive for <i>C. albicans</i>	1	0
Positive for <i>C. neoformans</i>	1	2
Negative	1	1

<sup>a</sup> Sabouraud dextrose agar and Mycosel agar slants.

(one sputum specimen and one urine specimen) in which *C. neoformans* was detected on birdseed agar only, the nonpigmented colonies of *C. albicans* greatly outnumbered the few pigmented colonies of *C. neoformans* (Fig. 1). The Sabouraud dextrose agar slants from these cultures were overgrown with *C. albicans*, which prevented colony identification of *C. neoformans*. *C. albicans* and *C. neoformans* were the only two yeasts isolated. Bacterial overgrowth was not seen on birdseed agar.

The concept of using selective media for isolating specific pathogens is not new to microbiologists but has rarely been used for fungal pathogens. In one selective medium described by Vogel, urease production was detectable as a pink halo around colonies of *C. neoformans*, but no such halo was formed around colonies of *C. albicans* (11). The combination of Littman oxgall agar (which inhibits saprophyte growth) with an extract from *G. abyssinica* seeds has also been described but not applied in a clinical microbiology setting (1). Likewise, a medium containing inositol, urea, and caf-

feic acid, while very successful for identifying *C. neoformans*, has not been used in a clinical microbiology setting as a primary isolation plating medium (4). Birdseed agar supplemented with antibiotics or with biphenyl (to inhibit hyphomycetes) has been used as a primary medium to enhance detection of *C. neoformans* (9, 10). In one study (10), seven patients were culture positive for *C. neoformans*; of these, six had positive sputum, urine, or stool cultures. In that study conventional medium was not used; therefore, the true utility of the birdseed agar (inhibiting bacterial contamination and providing differentiation from other yeasts) was not defined. However, some cultures had massive growth of *C. albicans* on birdseed agar that did not interfere with the detection of *C. neoformans* on birdseed agar by its production of brown pigment. Major texts on the laboratory diagnosis of fungal disease have not recommended this selective approach (2, 3, 6, 7). This may be because prior to the advent of AIDS, the combination of relatively silent pulmonary cryptococcosis and florid oral candidiasis was uncommon.

We incubated the birdseed plates at 30°C (unlike Staib et al., who used 26°C) (8–10), a temperature which permits the growth of all *Cryptococcus* species and other yeasts. Yeasts other than *C. neoformans* may rarely produce brown-pigmented colonies; other *Cryptococcus* species may produce a light brown pigmentation on a medium containing caffeic acid (4), and one isolate of *Trichosporon beigelii* also produced light brown pigmentation in a caffeic acid-ferric citrate test (13). In addition, rare strains of *C. neoformans* may not produce pigmented colonies on birdseed agar (10), and bacterial overgrowth has also been associated with failure to produce pigment (9). In view of this, presumptive identification is possible only with birdseed agar. It is interesting to speculate whether a firm identification may be possible directly from birdseed agar used as the primary isolation medium and incubated at 37°C. Growth at 37°C is virtually universal (though not optimal) in *C. neoformans* var. *neoformans* (virtually all cryptococcal infections in patients with

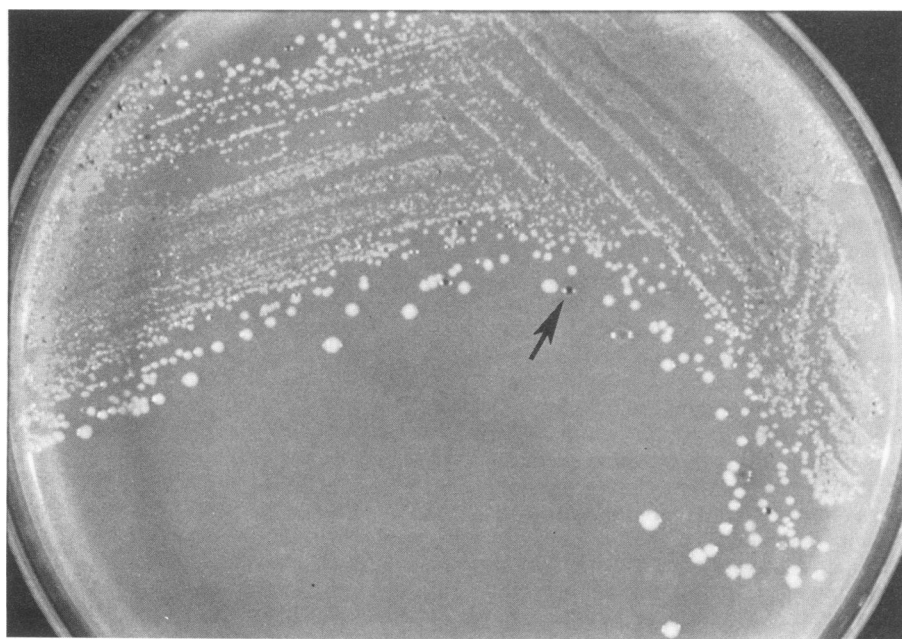


FIG. 1. A small number of pigmented colonies of *C. neoformans* (indicated by an arrow) in the presence of a large number of *C. albicans* colonies (not pigmented) on birdseed agar.

AIDS are caused by this variety); however, most other cryptococcal species and many other yeasts do not grow at 37°C (7). Therefore, incubation of the birdseed plate at 37°C might increase selectivity better by inhibition of most urease-producing yeasts. However, we know of no data on the performance of birdseed agar at this higher temperature.

We believe that positive cultures of *C. neoformans*, particularly from sputum, may have a major beneficial impact for particular patients with early cryptococcal disease in that treatment can be initiated early. We recommend the routine use of birdseed agar for sputum and urine fungal culture to facilitate the isolation of *C. neoformans*. Birdseed agar supplemented with antibiotics may be needed for specimens heavily contaminated with bacteria to prevent overgrowth on the birdseed agar plate.

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